LABEL-FREE DIELECTRIC DETECTION OF DNA HYBRIDIZATION WITH NANOGAP JUNCTIONS

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Abstract

A label-free dielectric detection of DNA hybridization with a nanogap junction is described. Nanogap junctions are fabricated with a novel spacer process technology and the change in capacitance of DNA within the nanogap after hybridization is measured. The 70% change of capacitance after hybridization is accomplished at 100 Hz for 10^{-5} *mol/L* concentrations of target DNA.

Keywords: Label-free detection, nanogap junction, dielectric spectroscopy, DNA hybridization

1. Introduction

Fluorescent, electrochemical, magnetic, radioisotopic, and enzymatic methods have been investigated extensively to detect DNA hybridization. However these methods are cumbersome and time consuming because of the need for DNA labeling. Therefore, label-free dielectric detection techniques of DNA hybridization have been investigated by many researchers. Generally, dielectric measurements in highly conductive liquids are difficult due to the electrode polarization caused by an electrical double layer formation on the electrodes. The potential difference in the solution due to the double layer causes a parasitic capacitance (*i.e.* electrode capacitance), which cannot be eliminated with conventional two-probe measurements. However, when two opposing electrodes are separated by nanoscale distances, the double layers overlap. The double layer thickness is the order of $3/\kappa$ to $4/\kappa$, where κ is Debye parameter [1]. For example, $1/\kappa$ is around 10 nm in 1 mM, 1:1 electrolyte solution at 25°C, so that double layer extends 30 ~ 40 nm into solution. The double layers can overlap in gaps less than 50 nm, decreasing the potential difference in solution. In this situation, the net ion densities are zero in most regions even around the electrode so that parasitic capacitance due to double layer can be reduced dramatically. Hence, the nanogap junction can achieve relatively high detection sensitivity in dielectric measurement without the four-electrode probe method. Based on this idea, the differences between dielectric properties of single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) are measured by the nanogap junction for the direct detection of DNA hybridization.

2. Experimental

Nanogap junctions are employed to improve the detection sensitivity of DNA hybridization. In order to fabricate a 50 nm gap between two polysilicon electrodes (nanogap junctions), a novel spacer process technology is used to attain nanoscale features which can not be achieved with conventional photolithography. The details of

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fabrication process for DNA sensors are described in [2]. During dielectric measurements, the two polysilicon layers act as electrodes, so that the dielectric properties of DNA located between them can be measured. Fig. 1 shows a cross-sectional SEM image showing the 50 nm gap between two polysilicon electrodes. Fig. 2(a) and (b) schematically show ssDNAs and dsDNAs on the nanogap junction, respectively. The ssDNA and the dsDNA are represented as a random coil configuration and a rod-like configuration, respectively, since ssDNA is considered more flexible than dsDNA.

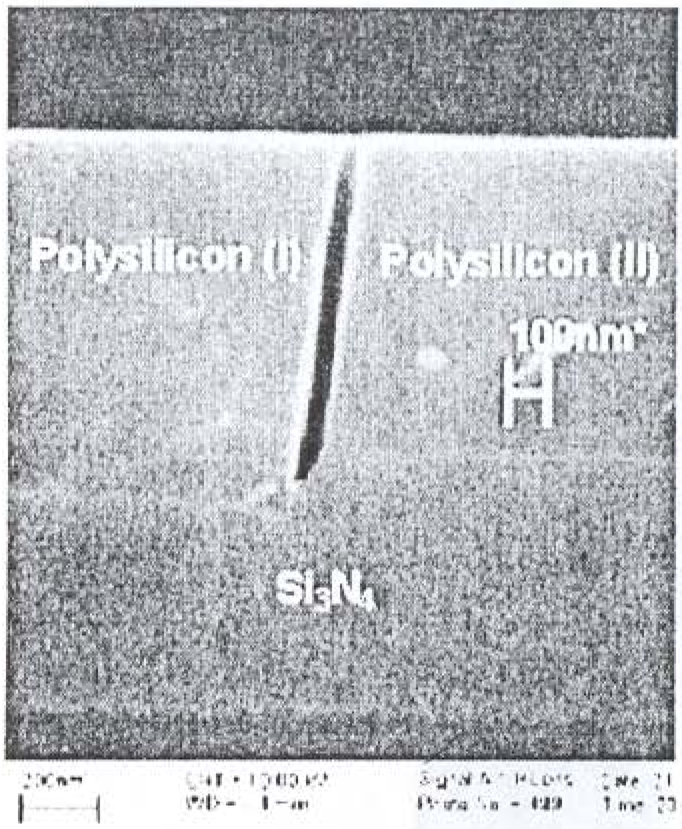

Fig. 1. A cross-sectional SEM image of a nanogap junction: The height of polysilicon is 1 μm and the width of nanogap is 50 nm.

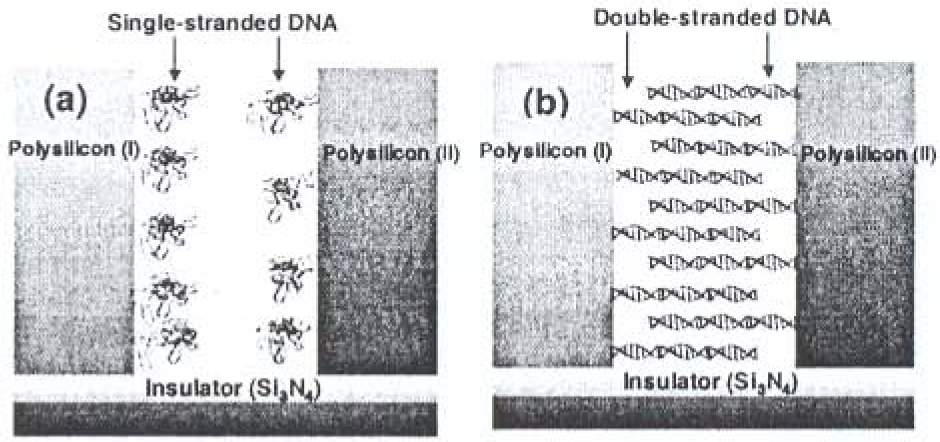

Fig. 2. Schematic diagrams of devices (a) with ssDNAs and (b) with dsDNAs.

Table I Materials in experiment

Item	Description
SAM	(3-Aminopropyl)triethoxysilane
DNA for immobilization	Poly T and poly G (35mer)
Buffer solution for immobilization	0.1M/L N-methylimidazole buffer containing 0.1M/L EDC (pH 6)
DNA for hybridization	Poly A and poly C (35mer)
Buffer solution for hybridization	300mM/L NaCl + 30mM/L sodium citrate
Washing solution	PBS

The materials used in this experiment and the details of the experimental procedures for monolayer formation, immobilization and hybridization are described in Table I and a previous report [2]. The dielectric properties are measured after DNA immobilization and DNA hybridization. In dielectric measurement, the permittivity and dissipation factor of DNA are investigated at frequency range of 100 Hz to 1 MHz, 0 V_{DC} bias and 25 mV_{AC} signals.

3. Results and Discussion

The changes of dielectric properties after hybridization step at different frequencies using different homonucleotides are shown in Fig. 3. So far, the maximum changes (60 ~ 70%) of capacitance are observed at 100 Hz after hybridization as shown in Fig. 3(a) and Fig. 3(b). The capacitance increases as frequency decreases and the relaxation frequency decreases after hybridization as expected since the persistence length of dsDNA is generally greater than that of ssDNA due to its unique double helix structure. In terms of capacitance change after hybridization, the complementary pairs of thymine(T)-adenine(A) and guanine(G)-cytosine(C) base show a significant increase and the non-complementary pairs of thymine(T)-guanine(G) and thymine(T)-cytosine(C) base show an insignificant change as expected.

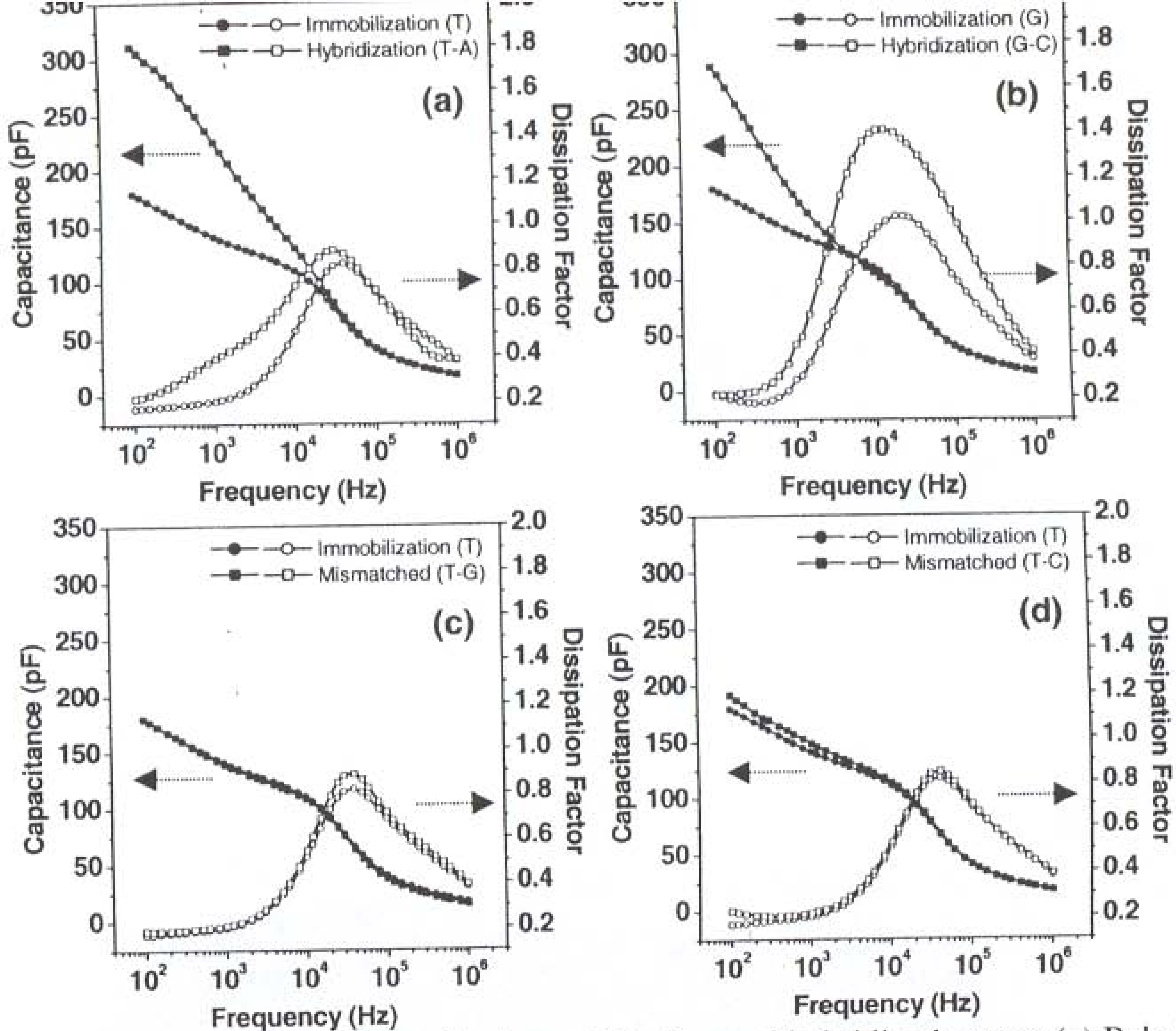

Fig. 3. Results of dielectric measurements after immobilization and hybridization step (a) Poly T and poly A, (b) poly G and poly C, (c) poly T and poly G and (d) poly T and poly C. The concentrations of poly T, poly A, poly G, and poly C are 1x10^{-5} *mol*/L.

To verify the relationship between capacitance change and hybridization, a nanogap junction device with immobilized poly T is treated with poly G and poly C instead of poly A. Fig. 3(c) and Fig. 3(d) show that there are no significant dielectric property changes in non-conjugate pairs. These results demonstrate that hybridization events are easily detectible without labeling.

4. Conclusion

A label-free DNA hybridization detection technique is accomplished by batch fabricated nanogap junctions using a novel spacer process technology. With the nanogap junction, a 70% change of capacitance after hybridization is measured at 10(Hz with 10^{-5} *mol*/L concentration of target DNA. The current system is mass producible as an array on a chip, allowing massively parallel label-free DNA hybridization detection with a high density nanogap junction arrays. The nanogap junction arrays can create an ultra-fast genotyping chip since it is relying on dielectric behaviors of biomolecular structural change.

References

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